REMARKS

According to the Office Action dated June 15, 2004, claims 7-21 were pending and claims 9, and 13 to 16 and 19 were withdrawn from consideration. In the present Amendment, claims 11 and 19 have been canceled without prejudice. Applicants reserve the right to prosecute the subject matter of claims 11 and 19 in one or more continuation, divisional or continuation-in-part applications. Claim 12 has been amended to correct its dependencies in view of the cancellation of claim 11. Claim 18 has been amended; support for the amendment to claim 18 can be found in the specification as filed at page 30, lines 13-21. Claim 21 has been amended. Support for the amendment can be found in the specification as filed at page 59, line 35 to page 61, line 17. Claim 22 has been added; support for new claim 22 can be found in the specification as filed at page 6, line 35 to page 7, line 1.

The Withrawal of Claim 19

According to the Office Action of June 15, 2004, claim 19 is withdrawn from consideration because claim 19 is allegedly drawn to a non-elected invention. Claim 19 is directed to genetically manipulated, replication competent, infectious virus of the paramyxoviridae family wherein the virus genome comprises a modification wherein the modification is selected from an insertion, substitution, or deletion of an open reading frame encoding a viral gene product. In the Restriction Requirement of June 17, 2003, Applicants were required to elect a restriction group from Group I directed to recombinant paramyxoviridae and Group II directed to methods of making recombinant negative stranded RNA viruses. In their response of July 9, 2003, Applicants elected to prosecute claims of Group I. Applicants respectfully assert that claim 19 belongs in Group I and should thus be examined with the other elected claims.

The Rejection under 35 U.S.C. § 112, First Paragraph, Should Be Withdrawn

Claim 12 directed to vaccine formulations is rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the enablement requirement. In particular, the Examiner contends that Applicants have not demonstrated that any of the modified RSV

disclosed in the application would be effective as vaccine against RSV infections. In particular, it is argued that the art does not presently accept a particular animal model as predictive of human responses to RSV vaccines. Applicants respectfully disagree because RSV infection in chimpanzees is an accepted animal model for RSV infections in humans and efficacy of vaccines of the invention has been shown in chimpanzees.

Dudas *et al.*, 1998, Clinical Microbiology Reviews 11:430-439 (cited in the Office Action; "Dudas") states, *e.g.*, at page 434, left column, 2nd full paragraph: "[a]s a model for immunization of young infants who have maternally derived RSV antibody, chimpanzees were infused with RSVIG, . . . " Thus, RSV infection in chimpanzees is accepted by the skilled artisan as a model for RSV infection in humans.

That compositions such as the compositions taught and claimed in the present application can be successfully used to protect chimpanzees from RSV infection has been shown in Teng et al., 2000, Journal of Virology 74(19): 9317-9321 ("Teng," attached as Exhibit A). In their study, Teng tested the protective efficacy of recombinant RSV with an inactivated NS1 or M2-2 gene (designated rA2ΔM2-2 and rA2ΔNS1, respectively) in the upper and lower respiratory tracts of chimpanzees. Attenuation and immunogenicity of rA2ΔM2-2 and rA2ΔNS1 are shown in Table 1 at page 9319 of Teng and protection against challenge with wild type RSV is demonstrated by the data shown in Table 2 at page 9319 of Teng.

Applicants respectfully invite the Examiner's attention to section 2164.02 of the MPEP:

> "[...] if the art is such that a particular model is recognized as correlating to a specific condition, then it should be accepted as correlating unless the examiner has evidence that the model does not correlate."

As RSV infection in chimpanzees is an art accepted model for RSV infection in humans and efficacy of vaccines of the invention has been shown in chimpanzees (see, *e.g.*, Teng), the presently claimed invention meets the requirements under 35 U.S.C. § 112, first paragraph.

The Examiner cites Prince et al., 2000, Journal of Virology, 74(22): 10287-10292 ("Prince") to support the contention that there are not effective anti-RSV vaccines for humans. Prince briefly summarizes the problems associated with replicating RSV vaccines

(first paragraph at page 10287). These problems, however, have been overcome by the present invention as demonstrated by the successful application of the vaccines of the present invention in chimpanzees (see Teng). Further, Prince describes the efficacy and safety of a subunit vaccine, the extramembrane domains of the F and G glycoproteins (FG). Thus, Prince is concerned with non-replicating RSV vaccines and can therefore not be compared with the replicating RSV vaccines of the present invention.

The Examiner further cites Tang et al., 2003, Journal of Virology 77(20): 10819-10828 ("Tang") to support the contention that there are not effective anti-RSV vaccines for humans. Tang states at page 10820, left column, 2nd full paragraph, that "Food and Drug Administration-approved vaccines for RSV, hPIV3, or hMPV are not currently available." However, there is no legal requirement that FDA approval is a condition for patentability. *In re Brana*, 34 U.S.P.Q.2d 1437, 1442 (Fed. Cir. 1995); *see also Scott v. Finney*, 34 F.3d 1058, 1063 (Fed. Cir. 1994).

Applicants further submit that the description found in the specification as filed is adequate and enabling for vaccine compositions. Under Section 112, it is not fatal that a certain amount of experimentation may be required to adapt the invention to a specific purpose, provided the experimentation is routine. In re Wands, 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988). Moreover, considerable amount of experimentation is permitted if it is merely routine or the specification provides a reasonable amount of guidance and direction to perform such experimentation. In re Jackson, 217 U.S.P.Q. 804, 807 (PTO Bd. Pt. App. Int. 1982).

Applicants submit that the specification contains an adequate description of the invention to enable the claims as currently pending. The provisions of Section 112, first paragraph, require that the description "enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same . . . (emphasis added). Applicants submit that any person skilled in the art of molecular biology can readily construct the recombinant viruses for use in vaccines as claimed by use of knowledge common in the art and in view of the teaching of the present specification.

Further, Applicants respectfully point out that procedures for testing a vaccine are routine in the art, and that the skilled artisan would be able to determine without undue experimentation which of the vaccines covered by the pending claims confer immunity to a subject when administered as a vaccine. In the context of this argument, the Applicants

would like to direct the Examiner's attention to *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (citing *In re Angstadt*, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19 (CCPA 1976)):

> " 'The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.' "

Thus screening procedures to test the vaccines of the invention for their potential to protect against viral infections and to confer immunity to a particular pathogen to a subject should not be considered undue experimentation since such procedures are well-known to the skilled artisan.

Further, Applicants respectfully point out that even though the present rejection was made under 35 U.S.C. 112, first paragraph, the gravamen of the Examiner's rejection is that the claimed compositions lack utility as vaccines. Similarly in *In re Brana*, 34 U.S.P.Q.2d 1437 (Fed. Cir. 1995), the Board had affirmed a final rejection under Section 112, 1st paragraph, of claims covering certain compounds asserted to be useful as anti-tumor substances because it was alleged that the specification was non-enabling since it did not sufficiently establish that the claimed compounds had a practical utility, *i.e.*, as anti-tumor agents. 34 U.S.P.Q.2d at 1439.

The Federal Circuit emphatically reversed the Board's decision. First, it explained the legal standard for compliance with the relevant Section 112 requirement, explaining that "unless there is reason to doubt the objective truth of the statements contained [in the specification] which must be relied on for enabling support", a specification's disclosure "must be taken as in compliance with the enabling requirement." *Id.* at 1441 (emphasis in the original). Further, the *Brana* Court made clear that the Patent and Trademark Office has the initial burden of challenging a presumptively correct assertion of utility; evidence must be presented that those of skill in the art would doubt the disclosure. Only then must the applicant provide rebuttal evidence.

Second, the Federal Circuit explained that even if one of skill in the art would have questioned the asserted use of the compounds, all applicants need do to overcome the rejection is to proffer sufficient evidence to convince one skilled in the art of the asserted utility. *Id.* at 1441.

In the *Brana* situation, the Court found that the Patent and Trademark Office had not met its initial burden. Further, the Court held that even if the Patent and Trademark Office had met its burden, the evidence proffered was clearly sufficient to meet the statutory requirement. As explained by the Court:

> We hold as we do because it is our firm conviction that one who has taught the public that a compound exhibits some desirable pharmaceutical property in a standard experimental animal has made a significant and useful contribution to the art, even though it may eventually appear that the compound is without value in the treatment of humans. *Id.* at 1442 [quoting *In re Krimmel*, 292 F.2d 948, 953 (CCPA 1961)].

Applicants respectfully point out that the *in vivo* chimpanzee protection data in Teng demonstrate that the claimed compositions have the asserted utility.

Applicants therefore respectfully request that the rejection under 35 U.S.C. 112, first paragraph, be withdrawn.

In their previous response of March 24, 2004, Applicants submitted in support of their position that the claimed compositions are enabled a publication that was co-authored by the inventor Hong Jin (Cheng *et al.*, 2001, Virology 283:59-68; "Cheng;" attached as Exhibit 1). Cheng shows that a recombinant RSV lacking the M2-2 open reading frame (rA2ΔM2-2) is attenuated in African green monkey and provides protection against infection with the wild-type virus (see Cheng, at page 63, Table 2 and page 64, Table 3). Applicants argued that because compositions that were taught and claimed in the present application were shown to be effective in an animal model system the claims should not be rejected for lack of enablement.

The Examiner found Applicants argument unpersuasive because there is allegedly no art-accepted model for testing RSV vaccines that is predictive of human responses to the RSV vaccines. The Examiner is respectfully reminded that the Court of Appeals for the Federal Circuit has held that testing for the full safety and effectiveness of a product is more properly left to the Food and Drug Administration and the requirements under the law for obtaining a patent should not be confused with the requirements for obtaining government approval to market a particular drug for consumption. *In re Brana*, 34 U.S.P.Q.2d 1437, 1442 (Fed. Cir. 1995); *see also Scott v. Finney*, 34 F.3d 1058, 1063 (Fed. Cir. 1994).

Claims 7, 8, 10-12, 17, 18, 20, and 21 directed to genetically manipulated, infectious paramyxoviruses and vaccine formulations, respectively, are rejected under 35 U.S.C. 112, first paragraph, as allegedly being non-enabled. In particular, the Examiner argues that the effect of a particular deletion or addition to the genome of the claimed virus is unpredictable and the examples provided by the Applicant are not commensurate in scope with the breadth of the claims. Further, the Examiner argues that Applicants do not provide sufficient guidelines for identifying insertions and deletions that result in the desired functional characteristics of the virus. Applicants respectfully disagree because the specification teaches how to make the viruses with an insertion or deletion or substitution of an open reading frame. The specification as filed further teaches how infectious, replication-competent viruses can be identified from among the genetically manipulated viruses. Thus, the skilled artisan equipped with the teachings of the present specification would be able to make and use the claimed viruses without more than routine testing.

THE LEGAL STANDARD

The test for enablement is whether one reasonably skilled in the art could make or use the invention, without undue experimentation, from the disclosure in the patent specification coupled with information known in the art at the time the patent application was filed. U.S. v. Telectronics Inc., 857 F.2d 778, 8 USPQ2d 1217 (Fed. Cir. 1988). In fact, well known subject matter is preferably omitted. See Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1384 (Fed. Cir. 1986) ("a patent need not teach, and preferably omits, what is well know in the art."). Further, one skilled in the art is presumed to use the information available to him in attempting to make or use the claimed invention. See Northern Telecom, Inc. v. Datapoint Corp., 908 F.2d 931, 941 (Fed. Cir. 1990) ("A decision on the issue of enablement requires determination of whether a person skilled in the pertinent art, using the knowledge available to such a person and the disclosure in the patent document, could make and use the invention without undue experimentation."). These enablement rules preclude the need for the patent applicant to "set forth every minute detail regarding the invention." Phillips Petroleum Co. v. United States Steel Corp., 673 F. Supp. 1278, 1291 (D. Del. 1991); see also DeGeorge v. Bernier, 768 F.2d 1318, 1323 (Fed. Cir. 1985).

Undue experimentation is experimentation that would require a level of ingenuity beyond what is expected from one of ordinary skill in the field. Fields v. Conover, 170

USPQ 276, 279 (CCPA 1971). The factors that can be considered in determining whether an amount of experimentation is undue have been listed in *In re* Wands, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Among these factors are: the amount of effort involved, the guidance provided by the specification, the presence of working examples, the amount of pertinent literature and the level of skill in the art. The test for undue experimentation is not merely quantitative, since a considerable amount of experimentation is permissible, so long as it is merely routine. *Id.*

Further, while the predictability of the art can be considered in determining whether an amount of experimentation is undue, mere unpredictability of the result of an experiment is not a consideration. Indeed, the Court of Custom and Patent Appeals has specifically cautioned that the unpredictability of the result of an experiment is not a basis to conclude that the amount of experimentation is undue in *In re Angstadt*, 190 USPQ 214 (CCPA 1976):

> [If to fulfill the requirements of 112, first paragraph, an applicant's] disclosure must provide guidance which will enable one skilled in the art to determine, with reasonable certainty before performing the reaction whether the claimed product will be obtained, ... then all "experimentation" is "undue" since the term "experimentation" implies that the success of the particular activity is uncertain. Such a proposition is contrary to the basic policy of the Patent Act. *Id.* at 219.

THE INSTANT SPECIFICATION PROVIDES AMPLE GUIDANCE TO THE SKILLED ARTISAN FOR MAKING AND USING THE CLAIMED VIRUSES

The instant specification, together with information which was readily available to the skilled artisan at the time the instant application was filed, provides a disclosure which fully enables the claimed viruses.

Applicants respectfully point out that the specification as originally filed provides ample guidance for how to make and use the genetically manipulated, infectious viruses. Indeed, the specification as filed teaches how to introduce mutations, such as insertions, deletions and substitutions of open reading frames, into the viral genome and how to evaluate the effect of the mutation on the viability of the mutated virus. Thus, the skilled artisan would know how to generate the claimed viruses without undue experimentation. However, the specification does not stop at teaching how to generate mutant viruses and how to select the claimed viruses, the application as filed also describes strategies for mutating viruses that

will yield the claimed viruses. Further, the specification as filed discloses an abundance of different mutant viruses that were actually generated, thereby providing guidelines for the skilled artisan of how to generate the claimed viruses.

The invention relates to the generation of non-segmented negative-stranded RNA viruses entirely from a cDNA encoding the viral genome (see, *e.g.*, page 12, line 36 to page 13, line 3 of the specification as filed). This technology provided for the first time the possibility to genetically engineer the viral genome of a non-segmented negative-stranded RNA virus (see, *e.g.*, page 14, lines 16-24, of the specification as filed). *E.g.*, section 6, beginning at page 30, describes the rescue of the virus. The specification further teaches that the viability of the rescued virus can be tested by, *e.g.*, plaque formation (page 42, lines 31-33).

Guidelines of how to arrive at recombinant non-segmented negative-stranded RNA viruses with an attenuated phenotype are provided in Section 5.4 beginning at page 20 of the specification as filed. Further, modifications of the RSV genome are described in section 6.2.1 beginning at page 38 of the specification as filed. For example, the specification as filed teaches that certain genes of RSV can be translocated from one position in the RSV genome to another to take advantage of the 3' to 5' gradient in virus gene expression to accomplish viral attenuation (see, *e.g.*, page 38, lines 29-34, and page 38, lines 4-15, of the specification as filed). Another type of mutations that is taught by the specification as filed are mutations in the cleavage site of the F protein to reduce the efficiency of its cleavage and thereby reduce virulence (see, *e.g.*, page 40, lines 6-14). Those types of mutations are only a few examples of strategies that are taught by the specification as filed for obtaining the claimed viruses.

Moreover, the specification as filed discloses many different mutant viruses that were actually made and analyzed. Substitution of the open reading frames of the G and F genes of one subgroup of RSV with the open reading frames of the G and F genes of another subgroup of RSV is described in section 8.1 beginning at page 49 of the specification as filed. Mutants of the L gene are described in section 9 at page 55 of the specification as filed. The effects of these mutants on the viability of the viruses are described in section 9.3.1 at page 58 to section 9.3.3 at page 59, and in Table II at page 62. These illustrative mutations and their effects on virus viability provide guidance for the skilled artisan to make and use the claimed

viruses. Mutant virus without the SH and/or M2-2 genes are described in section 10 beginning at page 59.

Applicants would like to direct the Examiner's attention to the situation in *Angstadt*. In *Angstadt*, the Applicants had developed a catalytic process using organometallic complexes. The board rejected the claims because the specification did not "give any information as to how the operative catalysts might be determined without undue experimentation." *Id.* at 216. The court, in rejecting the board's decision concluded that the skilled artisan armed with the specification and its 40 working examples would have been easily able to "determine which of the catalyst complexes within the scope of the claims work." *Id.* at 218. The court also held that the determination of which catalysts work does not "require ingenuity beyond that to be expected of one of ordinary skill in the art." *Id.* The *Angstadt* court further states that "the performance of trial runs using different catalysts is 'reasonable,' even if the end result is uncertain." *Id.* at 219. (emphasis added)

Angstadt further states:

> What the dissent seems to be obsessed with is the thought of catalysts which *won't* work to produce the intended result. Appellants have *enabled* those in the art to see that this is a real possibility, which is commendable frankness in a disclosure. Without undue experimentation or effort or expense the combinations which do not work will readily be discovered and, of course, nobody will use them and the claims do not cover them. The dissent wants appellants to make everything predictable in advance, which is impracticable and unreasonable.

Similarly, in the present application, simply because certain mutations in the genome of a non-segmented negative-stranded RNA virus will result in lethality of the virus, does not mean that the claimed invention is not enabled. As discussed above, the specification teaches how mutant viruses can be generated and how the replication competent, infectious viruses can be identified. The specification provides guidelines for types of mutations that will result in, *e.g.*, attenuated viruses. The specification also discloses an abundance of specific mutations that were made and analyzed to provide guidance to the skilled artisan for generating the claimed viruses.

The Examiner points to Bowie (Science 247:1306-1310; "Bowie") to support the position that the art of protein modification is unpredictable. As discussed above in the context of *Angstadt*, a claimed invention is enabled even if the outcome of a particular

embodiment is uncertain, as long as the testing involved is "reasonable." Testing whether a particular mutant virus is capable of forming plaques is certainly well within the ability of the skilled artisan and does not involve any unreasonable experimentation.

Applicants even describe a strategy to facilitate the identification of infectious and replicating viral mutants. In section 9.2, beginning at page 57, Applicants describe how the functionality of L gene mutants can be tested using the "minigenome" system. This system can be used to exclude mutants that would result in lethality of the virus caused by inactivity of the L gene.

Accordingly, Applicants respectfully request that the rejection of claims 7, 8, 10-12, 17, 18, 20, and 21 under 35 U.S.C. 112, first paragraph, be withdrawn.

Claim 21 directed to genetically manipulated, infectious paramyxoviruses is further rejected because Collins *et al.*, 1999, Virology 259:251-255, argue that the M2-1 open reading frame might be essential for virus viability. Without making any admissions and simply to expedite prosecution of the present application, claim 21 has been amended to recite that the deletion be a deletion of the M2-2 open reading frame.

Claims 7, 8, 10-12, 17, 18, and 20 directed to genetically manipulated, infectious paramyxoviruses and vaccine formulations, respectively, are rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the written description requirement. In particular, the Examiner argues that the limited number of examples does not provide sufficient support for the breadth of genetic variations permitted by the claims. It is further argued that the specification fails to provide necessary information regarding the structures of the viral proteins required to achieve the claimed functions.

THE LEGAL STANDARD

The test for sufficiency of written description is whether the disclosure of the application 'reasonably conveys to the artisan that the inventor had possession' of the claimed subject matter. *In re Kaslow*, 707 F.2d 1366, 1375, 217 U.S.P.Q. (BNA) 1089, 1096 (Fed. Cir. 1983); accord *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563; *see also*, *Ralston Purina Co. v. Far-Mar-Co, Inc.*, 772 F.2d 1570, 1575, 227 U.S.P.Q. (BNA) 177, 179 (Fed. Cir. 1985). The Court of Appeals for the Federal Circuit has repeatedly considered the

written description requirement and consistently found that exacting detail is not necessary to meet the requirement:

> If a person of ordinary skill in the art would have understood the inventor to have been in possession of the claimed invention at the time of filing, even if [not] every nuance of the claims is explicitly described in the specification, the adequate written description requirement is met.
> *In re Alton*, 76 F.3d 1168, 37 USPQ2d 1578 (Fed. Cir. 1996).

The criteria for determining sufficiency of written description set forth in Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112 ¶ 1, "Written Description Requirement" ("the Guidelines") (published in the January 5, 2001 Federal Register at Volume 66, Number 4, p. 1099-1111), specifies that:

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice (see (1)(a) above), reduction to drawings (see (1) (b) above), or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see (1)(c), above). *Id.* at p. 1106, column 3, *l.* 13-29.

> What constitutes a 'representative number' is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a 'representative number' depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. M.P.E.P. 2163(II)(A)(3)(a)(ii) and M.P.E.P. 2163.05(I).

Where the specification discloses any relevant identifying characteristics, *i.e.*, physical, chemical and/or functional characteristics sufficient to allow a skilled artisan to recognize the applicant was in possession of the claimed invention, a rejection for lack of written description under Section 112, first paragraph, is misplaced.

Furthermore, in accordance with the Guidelines, what is conventional or well known to one of skill in the art need not be disclosed in detail, and, where the level of knowledge and skill in the art is high, a written description question should not be raised.

THE INSTANT SPECIFICATION PROVIDES SUFFICIENT WRITTEN DESCRIPTION FOR THE CLAIMS

First, Applicants respectfully point out that the specification describes the claimed viruses in sufficient detail such that the skilled artisan would have understood that Applicants had been in possession of the claimed viruses. Applicants disclose the rescue of a non-segmented negative-stranded RNA virus from a cDNA encoding the viral genome, thereby making non-segmented negative-stranded RNA viruses accessible to recombinant DNA technology. As described throughout the specification, recombinant DNA technology can be used to genetically modify the viral genome and generate infectious, replicating viruses. In view of the specification, the skilled artisan would have known that Applicants were in possession of the claimed invention.

Applicants respectfully disagree with the Examiner that the number of examples is not representative for the scope of the claimed invention. As discussed in detail above in the section discussing the enablement requirement, the specification as filed discloses an abundance of viral mutants. The specification also discloses different types of mutants that fall within the scope of the claims, *e.g.*, attenuating mutants in section 5.4 beginning at page 20. The disclosed mutants in the working examples include substitutions, deletions, and substitutions of entire open reading frames. These mutations affected the G gene, the F gene, the M2-2 gene, the SH gene and the L gene. Thus, Applicants disclosed a wide range of different mutants within the scope of the claims.

With regard to the notion that specification fails to provide necessary information regarding the structures of the viral proteins required to obtain infectious and replicating viruses, Applicants point out that the specification teaches that the viruses be infectious and replicating and that this feature can be tested, *e.g.*, by plaque formation.

Accordingly, the rejection of claims 7, 8, 10-12, 17, 18, and 20 under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the written description requirement, should be withdrawn.

Claim 21 is rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the written description requirement. Without making any admission as to the merits of the rejection, Applicants assert that, in view of the present amendment, the rejection of claim 21 under 35 U.S.C. 112, first paragraph, should be withdrawn.

The Rejections under 35 U.S.C. § 102(a) Should Be Withdrawn

Claim 11 is rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by either Conzelmann *et al.*, 1994, J. Virol. 68(2):713-719 or Schnell *et al.*, 1994, The EMBO J. 13(18):4195-4203. Without making any admissions as to the merits of the rejection, Applicants respectfully point out that the present cancellation of claim 11 renders the rejection under 35 U.S.C. § 102(a) moot.

US Patent 6,033,886 to Conzelmann (the "'886 Patent")

Applicants further respectfully remind the Examiner that claims substantially corresponding to the claims pending in the present application were held to be patentable in the '886 Patent (see also Statement under 37 C.F.R. § 1.607(c) in the Response to Restriction Requirement of July 9, 2003 in connection with the present application).

Conclusion

Applicants respectfully request that the present remarks and amendments be entered and made of record in the instant application. An allowance of the application is earnestly requested. If any issues remain in connection herewith, the Examiner is respectfully invited to telephone the undersigned to discuss the same.

No fee is believed to be required for this response. However, should any fee be due, please charge the required amount to Jones Day Deposit Account No. 503013.

Respectfully submitted,

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Recombinant Respiratory Syncytial Virus That Does Not Express the NS1 or M2-2 Protein Is Highly Attenuated and Immunogenic in Chimpanzees

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Mutant recombinant respiratory syncytial viruses (RSV) which cannot express the NS1 and M2-2 proteins, designated rA2ΔNS1 and rA2ΔM2-2, respectively, were evaluated as live-attenuated RSV vaccines. The rA2ΔNS1 virus contains a large deletion that should have the advantageous property of genetic stability during replication in vitro and in vivo. In vitro, rA2ΔNS1 replicated approximately 10-fold less well than wild-type recombinant RSV (rA2), while rA2ΔM2-2 had delayed growth kinetics but reached a final titer similar to that of rA2. Each virus was administered to the respiratory tracts of RSV-seronegative chimpanzees to assess replication, immunogenicity, and protective efficacy. The rA2ΔNS1 and rA2ΔM2-2 viruses were 2,200- to 55,000-fold restricted in replication in the upper and lower respiratory tracts but induced a level of RSV-neutralizing antibody in serum that was only slightly reduced compared to the level induced by wild-type RSV. The replication of wild-type RSV in immunized chimpanzees after challenge was reduced more than 10,000-fold at each site. Importantly, rA2ΔNS1 and rA2ΔM2-2 were 10-fold more restricted in replication in the upper respiratory tract than was the *cpts*248/404 virus, a vaccine candidate that retained mild reactogenicity in the upper respiratory tracts of 1-month-old infants. Thus, either rA2ΔNS1 or rA2ΔM2-2 might be appropriately attenuated for this age group, which is the major target population for an RSV vaccine. In addition, these results show that neither NS1 nor M2-2 is essential for RSV replication in vivo, although each is important for efficient replication.

Respiratory syncytial virus (RSV) is the leading etiologic agent of serious pediatric viral bronchiolitis and pneumonia worldwide and is responsible for approximately 100,000 hospitalizations and 4,500 deaths among infants and children in the United States per annum (7, 14, 25). In addition, RSV infection can cause severe respiratory illness in the elderly (23) and in immunocompromised individuals (28). To date, an effective licensed vaccine for RSV is not available despite the pressing need for such an agent.

Since 1967, our laboratory has focused on developing a live-attenuated RSV vaccine for intranasal administration. By mimicking a natural infection, such a vaccine should stimulate both cellular and humoral immunity and would obviate the potentiated disease that was observed with certain nonreplicating or subunit vaccines (7, 16, 24, 27). The intranasal route also partially abrogates the immunosuppressive effects of maternal antibodies present in the sera of young infants and stimulates both local and systemic immunity (10).

A number of live-attenuated RSV vaccine candidates have been developed by biological or recombinant methods and evaluated in animals and humans (8, 15, 16, 29, 30, 32). The most promising biologically derived candidate, a cold-passaged (*cp*) temperature-sensitive (*ts*) virus called *cpts*248/404, was evaluated in RSV-naive 1- to 2-month-old infants and was found to be infectious, immunogenic, and protective against a second vaccine dose (33). However, some vaccinees experienced mild upper respiratory tract congestion, indicating that further attenuation is necessary. In addition, virus isolated late during the course of infection from a single vaccinee showed partial phenotypic reversion and loss of an attenuating mutation. Thus, our strategy to develop improved live-attenuated vaccine candidates has been (i) to use recombinant methods to combine attenuating mutations identified in a panel of biologically derived attenuated viruses including *cpts*248/404 and (ii) to develop new types of attenuating mutations by focusing on gene deletions which should be refractory to genetic reversion.

RSV is the prototype member of the *Pneumovirus* genus of the family *Paramyxoviridae*. Its genome is a single-stranded, negative-sense RNA of 15.2 kb that encodes 10 subgenomic mRNAs from which 11 proteins are translated. These proteins include the nucleocapsid N protein, phosphoprotein P, and large polymerase subunit L, which together comprise the minimal viral polymerase; fully processive transcription by the RSV polymerase requires the presence of the transcription antitermination factor M2-1 (6, 18, 19, 34). There are four envelope-associated proteins: the internal matrix (M) protein and three transmembrane surface proteins, namely, the attachment (G), fusion (F), and small hydrophobic (SH) proteins (7). Finally, RSV encodes two nonstructural proteins, NS1 and NS2, and also the M2-2 protein, whose status as structural or nonstructural is unknown. NS1 and M2-2 appear to have roles in RNA synthesis.

We previously described a reverse-genetics system for producing recombinant subgroup A RSV (rRSV) by coexpression of antigenomic RNA and the N, P, L, and M2-1 proteins from cotransfected plasmids (5). One application of this system has been to identify viral genes that can be deleted or silenced without ablating replication in vitro but are still necessary for

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virus replication in vivo (4, 26). Deletion of the SH gene resulted in a virus, designated rA2ΔSH, that replicated in vitro with an efficiency equal to or slightly better than that of wild-type rRSV (rA2) and which was moderately attenuated in mice and chimpanzees (4, 29). rRSV from which the NS2 gene was deleted, designated rA2ΔNS2, exhibited reduced growth kinetics and a reduced yield of infectious virus in vitro and was markedly attenuated in mice and chimpanzees (26, 29). Similar in vitro properties were noted for a recombinant bovine RSV from which the NS2 gene was deleted (2). These two deletion mutations are now being incorporated into recombinant live-attenuated vaccine candidates for clinical evaluation.

More recently, the M2-2 open reading frame was silenced in rRSV (rA2ΔM2-2, previously designated rA2-K5) by mutating each of the three potential translational initiation codons and inserting a translation termination codon in each of the three reading frames (1). A second research group made a comparable virus in which M2-2 was silenced by deletion of most of its open reading frame, which resulted in a virus that appeared to be phenotypically similar to rA2ΔM2-2 (20). The rA2ΔM2-2 virus exhibited increased plaque size, reduced growth kinetics (though the final titer was similar to that of the wild type), and a partial shift in RNA synthesis from RNA replication to transcription (1). Thus, the M2-2 protein appears to be a regulatory protein that negatively regulates transcription and positively regulates RNA replication. In addition, an rRSV was constructed from which the NS1 gene was deleted by the removal of nucleotides 122 to 630 in the antigenomic cDNA, resulting in the joining of the upstream nontranslated region of NS1 to the translational initiation codon of NS2. This virus, designated rA2ΔNS1, exhibited reduced RNA replication, plaque size, and growth kinetics and an approximately 10-fold lower yield of infectious virus in vitro (M. N. Teng and P. L. Collins, submitted for publication). Other paramyxoviruses encode proteins, such as the V protein of Sendai virus, that are not essential for replication in vitro. However, ablation of expression of V by recombinant Sendai virus results in attenuation in vivo (22). It was suggested that this protein functioned to antagonize some aspect of the mouse's innate immune system. More recently, the V protein of simian virus 5 was shown to block signalling for both type I and type II interferon responses (13). Any of the RSV "accessory" proteins, including the NS1, NS2, M2-2, SH, and G proteins, are candidates for antagonizing host immune mechanisms.

In the present study, we evaluated the rA2ΔM2-2 and rA2ΔNS1 viruses for replication, immunogenicity, and protective efficacy in the upper and lower respiratory tracts of chimpanzees, the only experimental animal in which RSV replication and virulence approaches that observed in humans. The rA2ΔM2-2 and rA2ΔNS1 viruses described above were constructed in the original version of the antigenomic cDNA described by Collins et al. (5). All recombinant viruses that have been constructed for vaccine purposes in our laboratory contain two types of modification to this background: (i) the introduction of a set of six translationally silent restriction markers in the L gene, called the sites mutations, and (ii) two amino acid substitutions in the F protein, called the HEK mutations, which make the recombinant virus identical at the amino acid level to the wild-type RSV A2 parent from which the *cpts*248/404 series of biological vaccine candidates was derived (21, 30). These mutations were shown to be phenotypically silent in chimpanzees (32). The rA2ΔNS1 virus used in this study was reconstructed in a sites-HEK background, in preparation for clinical evaluation, whereas the rA2ΔM2-2 virus is in the original genetic background, a difference that is not relevant for the present study (1, 30).

The rA2ΔNS1 and rA2ΔM2-2 viruses were administered individually to juvenile RSV-seronegative chimpanzees by combined intranasal and intratracheal inoculation, as described previously (11). Since both viruses were attenuated in vitro, we chose to inoculate the animals with 10^5 PFU per ml per site, which is a 10-fold higher concentration than that typically used to inoculate chimpanzees. To monitor virus replication in the upper and lower respiratory tracts, respectively, nasopharyngeal swabs and tracheal lavage samples were collected at intervals over 10 days postinfection and subsequently were assayed for virus titer. The mean peak virus titer was determined for each group (Table 1). The chimpanzees were monitored daily for rhinorrhea, a symptom of upper respiratory tract illness, and the mean peak score was determined for each group (Table 1). Due to the limited availability of RSV-seronegative chimpanzees, the number of animals per group was small, making it necessary to include controls from previous studies in which we had evaluated biologically derived RSV strain A2 (wild-type RSV A2), rA2, rA2ΔSH, rA2ΔNS2, and a recombinant version of the above-mentioned *cpts*248/404 vaccine candidate (rA2cp248/404) (Table 1).

Levels of replication of rA2ΔNS1 and rA2ΔM2-2 were reduced more than 2,200-fold and more than 2,800-fold, respectively, in the upper respiratory tract compared to that of rA2 (Table 1). Shedding of rA2ΔNS1 or rA2ΔM2-2 was detected sporadically and at a low level beginning 2 to 7 days postinfection, and each animal shed virus over a period of 3 to 8 days (data not shown). Thus, the recovered virus was not carried over from the initial inoculum but represented replication near the level of detection over a period of several days. In the lower respiratory tract, the level of replication of rA2ΔNS1 was reduced more than 17,000-fold compared to that of rA2, while rA2ΔM2-2 was undetectable at all time points (greater than 55,000-fold reduction). It is important to note that the dose of rA2ΔNS1 and rA2ΔM2-2 used was 10-fold greater than that of rA2. Furthermore, both viruses were more attenuated than rA2cp248/404, which was given at the same dose, particularly in the case of rA2ΔM2-2, which was not recovered from the lungs of infected chimps. In addition, both rA2ΔNS1 and rA2ΔM2-2 were unusual in being equally restricted in the upper and lower respiratory tracts. In the upper respiratory tract, each virus was approximately 10-fold more restricted than *cpts*248/404 and 175-fold more restricted than rA2ΔNS2. Since upper respiratory tract congestion was observed during clinical evaluation of the *cpts*248/404 virus in 1- to 2-month-old infants (33) and since infants of that age are obligate nose breathers, mutations that confer a level of restriction of replication in the upper respiratory tract greater than that of *cpts*248/404 would be desirable for inclusion in a live-attenuated vaccine virus. Animals receiving rA2ΔNS1 or rA2ΔM2-2 had slightly more rhinorrhea than those infected with rA2cp248/404, though still less than that of animals infected with a 10-fold smaller dose of rA2. While it is possible that the absence of NS1 or M2-2 resulted in a virus that retained a moderate level of virulence but replicated poorly, we think that this possibility is unlikely. Our experience is that quantitation of rhinorrhea and the comparison of such values from different studies performed at different times can be somewhat subjective and hence not completely reproducible. We anticipate that further evaluation, including clinical studies, will show that the amount of residual virulence associated with rA2ΔNS1 and rA2ΔM2-2 will reflect their greatly reduced replication.

Despite the highly restricted replication of these viruses, immunization with either rA2ΔNS1 or rA2ΔM2-2 induced a level of RSV-neutralizing antibody in serum that was within threefold of that induced by rA2cp248/404 (Table 1). Further-

TABLE 1. rA2ΔNS1 and rA2ΔM2-2 are highly attenuated in both the upper and lower respiratory tracts of chimpanzees but are highly immunogenic

Virus used to infect chimpanzees[a]	No. of animals	Dose[b] (per site, $\log_{10}$ PFU)	Mean peak virus titer[c] ($\log_{10}$ PFU/ml) ± SE (Duncan grouping)		Mean peak rhinorrhea score[d] (range, 0–4)	Mean neutralizing antibody titer in serum[e] (reciprocal $\log_2$)	
			Nasopharyngeal swab	Tracheal lavage		Day 0	Day 28
Wild-type RSV A2[f]	2	4.0	5.0 ± 0.35 (A)	5.5 ± 0.40 (A)	3.0	<3.3	11.2
rA2[g]	2	4.0	4.9 ± 0.15 (A)	5.4 ± 0.05 (A)	2.5	<3.3	10.5
rA2ΔSH[g]	3	4.0	4.6 ± 0.10 (A)	3.8 ± 0.31 (B)	1.0	<3.3	10.2
rA2ΔNS2[g]	4	4.0	3.8 ± 0.41 (B)	1.4 ± 0.29 (C)	1.0	3.4	10.6
rA2cp248/404[g]	4	5.0	2.5 ± 0.25 (C)	1.4 ± 0.37 (C)	0.8	3.4	10.6
rA2ΔNS1	4	5.0	1.6 ± 0.12 (D)	1.2 ± 0.43 (C)	2.0	<3.3	9.8
rA2ΔM2-2	4	5.0	1.5 ± 0.09 (D)	<0.7	1.8	<3.3	9.1

[a] All recombinant-derived viruses contain the sites and HEK mutations (see the text), except for rA2ΔM2-2.
[b] Chimpanzees were inoculated by the intranasal and intratracheal routes with the indicated amount of virus in a 1-ml inoculum per site.
[c] Nasopharyngeal swab samples were collected daily for 10 days, and tracheal lavage samples were collected on days 2, 5, 6, 8, and 10. Mean peak titers were calculated and assigned to statistically similar groups by Duncan's multiple-range test ($\alpha = 0.05$). Means in each column with different letters are significantly different.
[d] The amount of rhinorrhea was estimated daily and assigned a score (0 to 4) that indicated extent and severity. Scores indicate severe (4), moderate (3), mild (2), trace (1), or no (0) rhinorrhea. Shown are the mean peak scores.
[e] Serum RSV-neutralizing antibody titers were determined by a complement-enhanced 60% plaque reduction assay using wild-type RSV A2 and HEp-2 cell monolayer cultures incubated at 37°C. RSV-seronegative chimpanzee serum used as a negative control had a neutralizing antibody titer of <3.3 $\log_2$ reciprocal. Adult human serum used as a positive control had a neutralizing antibody titer of 11.4 $\log_2$ reciprocal.
[f] Historic controls from the study of Crowe et al. (10).
[g] Data from the study of Whitehead et al. (29).

more, animals previously infected with either rA2ΔNS1 or rA2ΔM2-2 were highly resistant to the replication of wild-type RSV administered intranasally and intratracheally 56 days postimmunization (Table 2). The levels of protection in both cases were similar in the upper respiratory tract and somewhat lower in the lower respiratory tract than that seen with *cpts*248/404, both in mean peak titer and in mean days of shedding.

The challenge in developing a live-attenuated RSV vaccine is to eliminate residual virulence without compromising immunogenicity. Observations to date indicate that the severity of RSV disease is closely related to the level of RSV replication in the respiratory tract. It is possible that one or more attenuating mutations that reduce virulence through another mechanism will be identified; indeed, it was hoped that deletion of one or more of the nonessential RSV proteins, such as those described in the present paper, might reveal such a virulence factor. However, a factor of this nature has not yet been identified for RSV. Thus, the present method for attenuating RSV is to reduce its level of replication, which unfortunately can reduce its immunogenicity due to the reduced production of antigen. The attenuating mutations that we have identified to date include (i) a set of five amino acid substitutions in the N, F, and L proteins that were identified in *cp*RSV and that confer attenuation in chimpanzees and humans (9, 16, 32); (ii) a series of amino acid substitutions in the L protein and a nucleotide substitution in the gene-start signal of the M2 gene, which were identified in biologically derived *ts* derivatives of *cp* RSV and which each confer the *ts* and attenuation phenotypes (12, 15, 21, 30); and (iii) deletion of individual or combinations of RSV genes such as the SH and NS2 genes (4, 26). Bovine RSV genes have also been used to confer attenuation based on host range restriction (3). Here, we add two additional knock-

TABLE 2. Infection of chimpanzees with rA2ΔNS1 or rA2ΔM2-2 induced significant protection against subsequent challenge with wild-type RSV A2 in the upper and lower respiratory tracts

Immunizing virus	Inoculum dose[a] ($\log_{10}$ PFU/ml)	No. of animals	Replication of RSV challenge virus at the indicated site[b]				Mean peak rhinorrhea score
			Nasopharynx		Trachea		
			Mean no. of days of shedding ± SE	Mean peak titer[c] ± SE	Mean no. of days of shedding ± SE	Mean peak titer ± SE	
rA2ΔNS1	5.0	4	2.8 ± 0.75	1.7 ± 0.46	1.0 ± 0.41	1.8 ± 0.73	1.0
rA2ΔM2-2	5.0	4	3.5 ± 0.87	2.3 ± 0.71	1.0 ± 0.71	1.7 ± 0.63	1.0
rA2ΔNS2[d]	4.0	4	ND	1.9 ± 0.30	ND	2.2 ± 0.77	1.0
*cpts*248/404[e]	4.7	2	3.5 ± 0.50	2.3 ± 0.25	0	<0.7	1.0
None[e]		2	8.5 ± 0.50	5.0 ± 0.35	6.0 ± 1.0	4.8 ± 0.30	3.0

[a] Each virus was initially administered at the indicated dose in a 1.0-ml inoculum given intranasally and intratracheally.
[b] On day 56, chimpanzees were challenged with wild-type RSV A2 administered at a dose of 10^4 PFU/ml in a 1.0-ml inoculum given intranasally and intratracheally. Nasopharyngeal swab samples were collected daily for 12 days, and tracheal lavage samples were collected on days 2, 5, 6, 8, and 12. ND, not determined.
[c] Mean peak titers ($\log_{10}$ PFU/ml) were calculated by using the peak virus titer achieved in each animal.
[d] Data from the study of Whitehead et al. (29).
[e] Historic control animals from the study of Crowe et al. (10) were used.

out mutations to the list, namely, the deletion of NS1 and the silencing of the M2-2 open reading frame.

Among the mutant viruses shown in Table 1, the order of increasing attenuation in seronegative juvenile chimpanzees was rA2ΔSH < rA2ΔNS2 < rA2cp248/404 < rA2ΔNS1 < rA2ΔM2-2. All viruses provided similar, high levels of protection against challenge with wild-type RSV (Table 2). Thus, rA2ΔNS1 and rA2ΔM2-2 each have the desired property of being slightly more attenuated than rA2cp248/404, the recombinant version of *cpts*248/404, which was slightly too reactogenic in RSV-naive 1- to 2-month-old infants, as mentioned above (33). The finding that rA2ΔM2-2 is slightly more attenuated than rA2ΔNS1 increases the chances that one of these viruses will have an optimal level of attenuation. The seronegative juvenile chimpanzee is somewhat less permissive to RSV replication and disease than is the RSV-naive human infant. Thus, whether rA2ΔNS1, rA2ΔM2-2, or both have an appropriate level of attenuation can be determined only by clinical trials with the target vaccine population, 1- to 2-month-old infants.

Deletion mutants should be extremely stable both in vitro and in vivo, thus making them attractive candidates for vaccine development. This property might be important in light of the finding that one infant who had been vaccinated with *cpts*248/404 shed virus that exhibited a partial reversion (33). A low level of genetic instability in an RSV vaccine likely would not be a problem in normal individuals, particularly considering the high prevalence of fully virulent wild-type RSV. However, vaccine virus might have prolonged replication in immunocompromised individuals. Thus, it would be desirable to engineer a recombinant vaccine virus to contain attenuating mutations that cannot revert.

Although the major target for an RSV vaccine is the 1- to 2-month-old infant, a second target is the elderly. The *cpts*248/404 vaccine candidate, which was insufficiently attenuated in the RSV-naive infant, was found to be overattenuated in the RSV-experienced adult (17). Thus, a live-attenuated vaccine for RSV-naive infants will need to be more attenuated than one for use in adults. Since the rA2ΔNS1 and rA2ΔM2-2 viruses are similar to *cpts*248/404 in their levels of replication, they likely will be too attenuated to be useful as an adult vaccine. However, each virus is appropriate for further evaluation as a pediatric RSV vaccine, either as currently constructed or with the inclusion of a single or a combination of additional attenuating mutations. It should be noted that if either candidate vaccine proves satisfactory, a partner subgroup B candidate can be rapidly generated by replacing the F and G glycoproteins (31).

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